

Quantitative and Temporal Relationships Between DNA Adduct Formation in Target and Surrogate Tissues: Implications for Biomonitoring

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DNA-carcinogen adducts offer a potential dosimeter for environmental genotoxins reaching the exposed individual. Because the target tissues for many chemical carcinogens are not readily accessible for monitoring adducts in humans, peripheral blood lymphocytes (PBLs) have served as surrogate sources of exposed DNA. Both benzo[a]pyrene (BaP) and benzo[b]fluoranthene (BbF) are widely distributed in the environment as components of complex mixtures, such as automobile exhaust, cigarette smoke, foods, water, and urban air. Thus, human exposure to these chemicals is widespread, and they probably contribute to overall human lung cancer risk. The interpretation of the results of such studies would be enhanced by an understanding of the pharmacokinetics of specific DNA adduct formation and persistence in both target and surrogate tissues. Polycyclic aromatic hydrocarbons (PAHs) were administered to male Sprague-Dawley rats IP at 100 mg PAH/kg body weight. Lung, liver, and PBL tissues were harvested 1, 3, 7, 14, 28, and 56 days after treatment. DNA was extracted from each tissue and ³²P-postlabeling analysis of DNA adducts with nuclease P1 enhancement was conducted. In all three tissues, BaP-DNA adducts exhibit a similar pattern, reaching a maximum at 3-4 days, followed by a decrease to 56 days. For BbF, the maximum DNA adduct levels in each tissue were between 5 and 14 days after injection. By 56 days after administration, the total adducts remaining in all tissues were measurable. Correlation analyses of the amount of DNA adducts in lung or liver compared to those found in the PBL of the same animals suggest a range of correlations ($R^2 = 0.67-0.83$). For BaP, DNA adducts in both liver and lung may be predicted by PBL DNA adduct levels. For BbF, adduct levels in PBLs directly reflect adduct levels in the liver and are less predictive of lung adduct levels. The collateral pharmacokinetics for DNA adduct persistence in lung, liver, and PBLs suggest that PBL adduct-based dosimetry may reflect patterns of adduction in other less accessible tissues. Thus, PBL DNA adducts may prove to be useful dosimeters for the delivered dose of DNA.

Introduction

DNA-carcinogen adducts may offer a potential dosimeter for environmental genotoxins reaching the exposed individual. Since the advent of sensitive techniques that can measure DNA adducts in human tissues at extremely

high levels of sensitivity, there has been numerous papers describing studies on DNA adducts in humans exposed to environmental agents. Industrial populations exposed to processes such as aluminum smelters (1), coke ovens (2), iron and steel foundries (3), and roofing (4), and populations exposed to cigarette smoke (5) and genotoxic agents have been studied (6). In all of these studies, peripheral blood lymphocytes (PBLs) have been used as the accessible target tissue in which to measure the formation of DNA adducts. Because the target tissues for many chemical carcinogens are not readily accessible for monitoring adducts in humans, PBLs have served as surrogate sources of exposed DNA. The interpretation of the results of such studies would be enhanced by an understanding of the pharmacokinetics of specific DNA adduct formation and persistence in both target and surrogate tissues.

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Benzo[*a*]pyrene (BaP) and benzo[*b*]fluoranthene (BbF) are bay-region-containing polycyclic aromatic hydrocarbons (PAHs) widely distributed in the environment as components of complex mixtures, found in automobile exhaust, cigarette smoke, foods, water, and urban air (7). PAHs are components of many emissions arising from the combustion of coal, petroleum fuels, and plant materials (7). The goals of this study were to examine time-course relationships between BaP and BbF administration and DNA adduct formation and persistence in accessible and target rat tissues and to identify novel DNA adducts formed in target tissues to relate to their mechanisms of metabolic activation in chemical carcinogenesis. This paper reviews previous work in this study, presents new data, and outlines new research directions.

Materials and Methods

Male Sprague-Dawley rats 21 days old were obtained from Charles River Breeding Laboratories (Morrisville, NC) and were housed three per cage. Rats were fed Purina lab chow and water *ad libitum*. Animals were maintained at 20–21°C, relative humidity 60–68%, on a 12-hr light-dark cycle. At the time of PAH administration, the animals weighed between 280 g and 405 g and were approximately 6 weeks old. PAHs were administered as a uniform suspension in sunflower seed oil in a single IP injection at 0.05 mL/10 g body weight to yield a final dose of 100 mg/kg. Three animals were used for each time point, and control animals received IP injections of the oil carrier alone.

Rats were anesthetized with Metofane, (methoxy-flurane, Pitman-Moore, Washington Crossing, NJ) at various times after PAH administration and approximately 10 mL of blood was removed by cardiac puncture. The rats were then sacrificed by cervical dislocation, and the lungs and livers were surgically removed and frozen at –80°C. DNA was isolated from tissues by a spermine precipitation method (8). Tissues from individual animals were analyzed separately.

³²P-Postlabeling Analysis of DNA Adducts

DNA (5 µg/adduct assay) was hydrolyzed to 3'-mononucleotides by digestion with micrococcal nuclease and spleen phosphodiesterase as previously described (9). The efficiency of adduct labeling relative to nonadducted nucleotides was enhanced by digestion with nuclease P1, as described by Reddy and Randerath (10). Adducts were labeled at the 5' position with polynucleotide kinase and γ-³²P-ATP. Radiolabeled adduct nucleotide bisphosphates were then separated by thin-layer chromatography as previously described (11). Two solvent systems were routinely employed. System 1 was D1 = 1 M sodium phosphate, pH 6.0, with overnight chromatography onto a 5-cm Whatman grade 17 Chr wick, followed by a wash with water; D3 = 3.5 M lithium formate, 7 M urea, pH 3.5, with development 6–8 cm onto a Whatman-grade 1 wick, followed by a wash with water; a wash with 20 mM Tris base,

and an additional wash with water; D4 = 1:1 isopropanol:4 N ammonia, with development 6–8 cm onto a Whatman-grade 1 wick; and D5 = 1.7 M sodium phosphate, pH 6.8, with development 6 cm onto a Whatman-grade 1 wick. System 2 was D1 = 1 M sodium phosphate, pH 6.0, with overnight development onto a 10-cm Whatman-grade 3MM Chr wick followed by a wash with water; D2 = 2.75 M ammonium formate, developed 1 cm; D3 = 4.5 M lithium formate, 7 M urea, pH 3.5, followed by a wash with water; D4 prewash = 1 cm development with 0.5 M Tris-HCl; D4 = 1.1 M lithium chloride, 0.5 M Tris-HCl, 7 M urea, pH 8.0, followed by a wash with water; and D5 = 1.0 M MgCl₂, with development onto a 3-cm Whatman-grade 3MM Chr wick, followed by a final wash with water. Separated adducts were visualized by autoradiography using DuPont Cronex Lightning Plus intensifying screens and Kodak XAR-5 X-ray film overnight at –80°C. Adduct levels were quantitated using the technique of Gupta (9).

Results

The single IP administration of BaP to rats produced a time-course relationship between BaP-DNA adduct formation, persistence, and decay over a 56-day period (Fig. 1). This relationship shows an expected early peak of DNA adduct formation and slow decline of adduct persistence and decay. Maximal DNA adducts present in lung, liver, and PBLs occur at approximately 3 days after a single IP administration. Lung and liver DNA is adducted in the pmole/mg DNA range, with PBL DNA adducted at one-third that seen with lung DNA. The three tissues show parallel DNA adduct formation, persistence, and decay time-course relationships over a 56-day period. At 56 days, residual DNA adducts in each tissue are clearly measurable up to 150 fmole/mg DNA.

The DNA adduct autoradiograms from postlabeling analysis revealed that each tissue produced unique patterns of DNA adducts and that there were several novel and as yet unidentified DNA adducts in both lung and liver DNA of these animals (12). Lung DNA contained the expected benzo[*a*]pyrene diol-epoxide (BPDEI)-dGuo DNA adduct with two slower migrating, major DNA adducts. Liver DNA also contained the expected BPDEI-dGuo adduct with a faster migrating, new adduct (data not shown).

To identify these new adducts, a series of BaP metabolites and synthetic standards were administered to rats and after 3 days lung, liver, and PBL DNA was isolated and examined for the presence of specific BaP-DNA adducts (12). The results of this analysis indicated that although several BaP metabolites/standards produced DNA adducts, only two produced DNA adducts that comigrated with DNA formed by administration of BaP alone. 9-Hydroxy-BaP produced the new slower DNA adducts seen in lungs from rats administered BaP, which was confirmed by co-chromatography of the two DNAs in several TLC solvent systems. *trans*-7,8-Dihydro-7,8-dihydroxy-BaP produced the new adduct observed in the liver of rats administered BaP as well as the BPDEI-dGuo DNA adduct.

Administration of BbF to rats at 100 mg/kg by a single IP administration produced a pattern of DNA adducts, that, unlike BaP, was similar in all three tissues (13). Quantitatively DNA adduct formation in the lung was approximately five times that found in the liver or PBLs. The peak of BbF-DNA adduct formation in all three tissues occurred between 5 and 14 days after administration (Fig. 2). Loss of DNA adducts in the lung was slower than that observed in the liver or PBLs with approximately 50% remaining after 56 days. Co-chromatography of DNA from BbF-treated rats with DNA reacted with the bay-region diol-epoxide of BbF (*trans*-9,10-dihydro-9,10-dihydroxy-BbF-11,12-oxide) indicated that almost none of

the BbF-DNA adducts in the rat was related to the bay-region diol-epoxide.

Discussion

BaP induces a wide range of tumors in the rat depending on the route of administration. Systemic administration produces mammary tumors; esophageal and forestomach tumors are produced by intragastric or oral administration (14-17). Subcutaneous administration of BaP produces both site of injection fibrosarcomas and mammary tumors; intratracheal instillation and lung implantation both produced lung tumors (18-22). BbF has

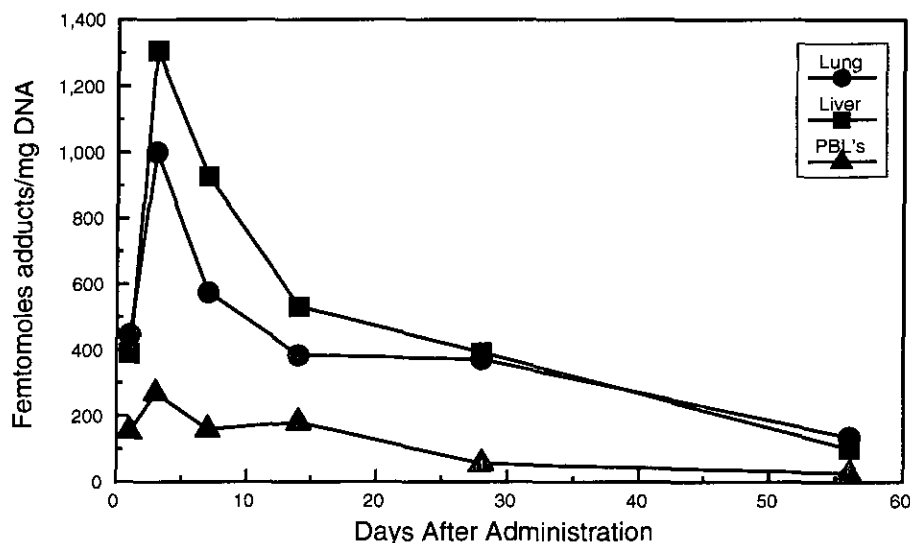


FIGURE 1. Accumulation, persistence, and decay of benzo[a]pyrene (BaP) DNA adducts in lung, liver, and peripheral blood lymphocytes from rats administered a single IP dose of 100 mg/kg BaP. Each point represents the mean of duplicate determinations on tissues from each of three rats.

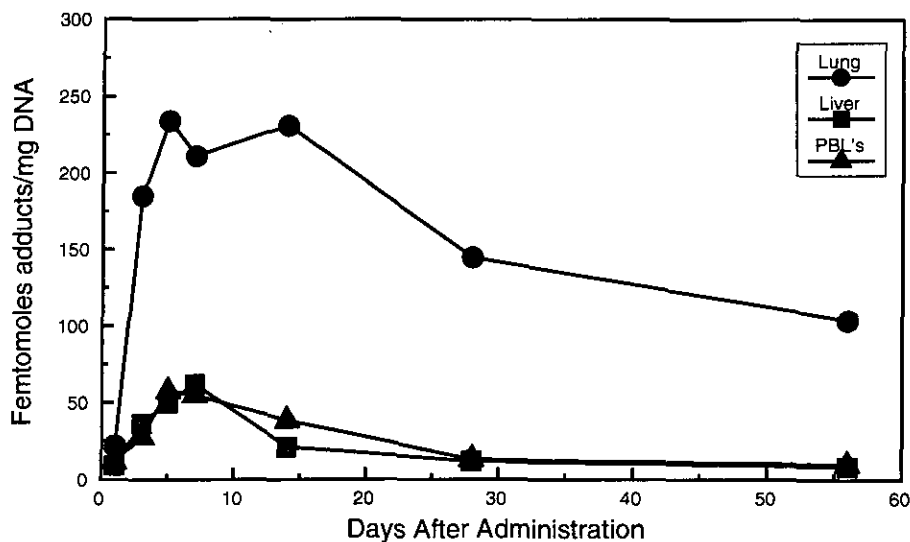


FIGURE 2. Accumulation, persistence, and decay of benzo[b]fluoranthene (BbF)-DNA adducts in lung, liver, and peripheral blood lymphocytes from rats administered a single IP dose of 100 mg/kg BbF. Each point represents the mean of duplicate determinations on tissues from each of three rats.

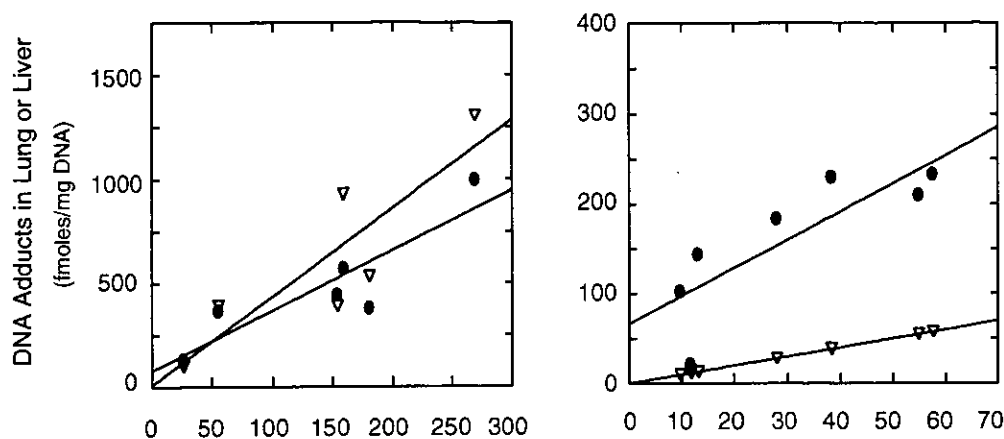


FIGURE 3. Correlation plots of DNA adducts in lung or liver compared to DNA adducts in peripheral blood lymphocytes based on all time points. (Left panel) benzo[a]pyrene; (right panel) benzo[b]fluoranthene; (●) lung, (▽) liver.

not been as widely studied in the rat. The only reported study is a lung implantation study that produced lung tumors (22). Thus, the respiratory tract is major target for both these PAHs in the rat.

The major question of the appropriateness of using available surrogate tissues such as PBLs to monitor internal target tissue dose for several PAHs has been answered. The BaP- and BbF-DNA adduct formation, persistence, and decay curves over a 56-day period for lung, liver, and PBL tissues are remarkably similar. Quantitative determination of this similarity can be obtained by examining the correlation coefficient between DNA adducts in PBLs and DNA adducts in lung or liver tissues over the complete time course study (Fig. 3). For BaP, the correlation coefficient between BaP-DNA adducts in PBLs and BaP-DNA adducts in lung or liver range from 0.75 to 0.78, a very strong correlation (Table 1). For BbF, the correlation coefficient between BbF-DNA adducts in PBLs and BbF-DNA adducts in lung is 0.67, whereas the correlation between DNA adducts in PBLs and liver is 0.83. This suggests that DNA adducts in PBLs can serve as surrogates for DNA adducts in internal organs for these PAHs with some confidence. The levels of DNA adduction in the PBLs of rats 56 days after treatment is comparable to that found in PBLs obtained from humans exposed to a variety of PAH-rich exposures including

those due to aluminum smelters, coke ovens, iron and steel foundries, roofing tar emissions, cigarette smoking, and psoriasis treatment with coal tar (Table 2). This places more credence on these correlations, as they were not totally based on comparisons at extremely high DNA adduction levels.

The partial identification of the novel BaP-DNA adducts that are further metabolites of *trans*-7,8-dihydro-7,8-dihydroxy-BaP and 9-hydroxy-BaP has prompted the synthesis of 4,5-difluoro-BaP, a fluorinated BaP blocked at the 4,5-bond or K-region (23). The literature prior to the postlabeling methodology suggested that 9-hydroxy-BaP may be further metabolized to a reactive form (possibly 9-hydroxy-BaP-4,5-oxide) that can bind to DNA, as indications of this adduct were observed in rat liver and lung (24-25). Interestingly, rat liver microsomal metabolism of 9-fluoro-BaP gives both *trans*-7,8-dihydro-7,8-dihydroxy-9-fluoro-BaP and *trans*-4,5-dihydro-4,5-dihydroxy-9-fluoro-BaP suggesting the 7,8 bond as a possible target for the second oxidation step of 9-hydroxy-BaP (39). Studies are underway to explore the

Table 2. Comparison of levels of PAH-DNA adducts in rat and human PBLs.

Species/exposure	Total DNA adducts /10 ⁸ nucleotides
Rat/BaP (after 56 days)	0.87
Rat/BbF (after 56 days)	0.33
Human/Finnish foundry workers (3) ^a	0.75-2.5
Human/Polish coke oven workers (2)	15.3
Human/aluminum smelter workers (1)	1.48-3.08
Human/psoriasis patients treated with coal tar (6)	0.18-9.4
Human/cigarette smokers (5)	8-61
Human/roofers (4)	0.1-9.6

Abbreviations: PAH, polycyclic aromatic hydrocarbon; PBL, peripheral blood lymphocytes; BaP, benzo[a]pyrene; BbF, benzo[b]fluoranthene.

^aWhite blood cells.

Table 1. Correlation (R^2) between PAH-DNA adduct levels in PBLs and PAH-DNA adduct levels in lung and liver tissues.^a

PAH	Lung	Liver
BaP	0.78	0.75
BbF	0.67	0.83

Abbreviations: PAH, polycyclic aromatic hydrocarbon; PBL, peripheral blood lymphocytes; BaP, benzo[a]pyrene; BbF, benzo[b]fluoranthene.

^aCorrelations based on values at all time points from data found in Figures 1 and 2. Correlation coefficients obtained from linear least squares regression analysis.

Table 3. Summary of apparent half-lives of PAH-DNA adducts in three rat tissues.^a

PAH	Lung	Liver	PBLs
BaP ^b	22 (29) ^c	13	16
BbF	18	8	8

Abbreviations: PAH, polycyclic aromatic hydrocarbon; PBLs, peripheral blood lymphocytes; BaP, benzo[a]pyrene; BbF, benzo[b]fluoranthene.

^aDetermined from the exponential decay curves of a log-linear plot. Values in days.

^bBased on benzo[a]pyrene diol epoxide 1-dGuo adduct.

^cBased on 9-hydroxy-BaP-DNA adducts.

identity of the two new DNA adducts of BaP using this fluorinated probe.

Our analysis of the pattern of DNA adducts from rats treated with BbF suggests that none of the adducts are related to the bay-region diol epoxide of BbF (*trans*-9,10-dihydro-9,10-dihydroxy-BbF-11,12-oxide). These results are in concert with those found by analyzing DNA from mouse epidermis treated with BbF (27). Although BbF is a strong mouse skin tumor initiator, the major mouse skin DNA adducts of BbF are not related to the bay-region diol epoxide.

Comparison of the half-lives of the DNA adducts in each tissue for each PAH suggested that PAH-DNA adducts persist longer in lung tissues than in liver or PBLs (Table 3). BaP-DNA adducts seem to persist twice as long as BbF-DNA adducts for each tissue, with maximal half-life times of 23 days recorded. The half-lives of these PAH-DNA adducts in PBLs are 8–16 days. Experimental determination of the lifetimes of lymphocytes in humans and mice have been studied. In women, there seems to be two populations of lymphocytes, one short-lived (3–4 days) and another larger fraction that are long-lived [530 days (28)]. Similar results have been found in mice with a short-lived fraction (3–5 days) and a larger longer lived fraction of 112 days (29). In the rat, two populations of lymphocytes have also been reported with lymphocyte lifetimes relatively longer than those found in the mouse (30,31).

These studies in experimental animals are crucial to developing the background for the use of PBLs as surrogate tissues for biomonitoring exposure of humans to genotoxic agents. They also provide a firm experimental basis for the identification of the structure of the DNA adducts being monitored and the techniques for observing these adducts in more complex human samples. The knowledge of the collateral pharmacokinetics of DNA adducts in target and surrogate tissues and the exact nature of the adducts is important information when relating levels of these DNA adducts to potential hazard outcomes from the exposure.

This work was supported in part by the U.S. Environmental Protection Agency, Cooperative Research Agreement CR 816185. The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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